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REVIEW

Current status of androgen receptor-splice variant 7 inhibitor niclosamide in castrate-resistant prostate-cancer

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Summary
Castrate-Resistant Prostate-Cancer (CRPC) is one of the most common malignancies occurring in men. Unfortunately, even if several recently approved agents clinically improved the outcome of CRPC patients, none of these is curative especially for a splice version of the Androgen Receptor (AR) AR-V7, which is a variant of the receptor constitutively activated and does not require the presence of androgens for the activation AR down-stream pathways. Since high AR-V7 expression is one of the most common features of CRPC, targeting this receptor variant is considered as one of the most promising strategies for treating this disease. Therefore anti-AR-V7 molecules could lead to a potential shift in paradigm in the treatment of CRPC. Niclosamide, an already FDA-approved anti-helminthic drug, was identified as a potent AR-V7 inhibitor in prostate cancer cells. Due to the recent positive preclinical results, niclosamide may be an interesting and novel type of targeted treatments for CRPC. This mini-review outlines the most recent pre- and clinical- data on the current status of niclosamide in the treatment of ARV7-positive CRPC patients.

Keywords Castrate-resistant prostate-Cancer · Androgen receptor · Androgen receptor splice variant 7 · Niclosamide

Introduction

Prostate cancer (PC) is one of the most common causes of cancer-related deaths worldwide, with approximately 164,690 new occurrences of the disease and 29,430 deaths in 2018 [1]. Although during the last years, inhibition of Androgen Receptor Signalling (ARS) as well as conventional chemotherapeutic agents, reported 5-year survival values as low as 29%, the end-stage of Castrate-Resistant Prostate-Cancer (CRPC) is still incurable [2–5]. Even if during the years, food and drug administration (FDA) approved several hormonal agents such as flutamide, bicalutamide and nilutamide,there was still a need for not having agonist activity against wild-type AR, not recruiting AR coactivators and consequently blocking the subsequent AR binding to DNA and acting as a transcription factor for the expression of androgen-dependent genes leading to tumour proliferation. This requirement brought to the development of new drugs that subsequently were defined as second-generation antiandrogens. Consequently, in 2012 enzalutamide and abiraterone acetate were FDA approved as second-generation antiandrogens [2, 3]. There are also other second-generation anti-androgens that are at various stages of preclinical and clinical development. The FDA approved also an immunotherapeutic agent Sipuleucel-T after the successful results of a phase III randomized-to control clinical trial (IMPACT) showing a decrease in the risk of death and an increase in median survival [4]. In this context, niclosamide, an FDA-approved anti-helminthic drug, generated positive results in the first preclinical studies [4, 5]. The aim of this mini-review is to summarize the first preliminary available data on niclosamide in PC. Finally, future directions will be discussed.

The molecular basis of tumour growth by androgen receptor signalling

Androgen receptor (AR) is a cytoplasmic ligand-dependent transcription factor, which directs the expression of specific

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genes involved in differentiation and sexual development. The main AR native ligand is testosterone, which is primarily synthesized by the Leydig cells in testies; androgens such as testosterone are under the regulation of a hormone produced by the anterior pituitary gland, the luteinizing hormone (LH), which in turn is regulated by gonadotropin-releasing hormone (GnRH). There are other endogenous androgens, such as dihydrotestosterone, androstenedione, androstenediol, DHEA, androsterone. Examples of synthetic androgens are methyltestosterone, nandrolone, metandienone, trenbolone, stranozolol and oxandrolone (Fig. 1).

As to the example of testosterone, inside vessels this androgen circulates bound to albumin and serum sex hormone-binding globulin (SHBG). Subsequently only the unbound form of SHBG enters cells [6, 7], which is a powerful metabolite- named 5α-dihydrotestosterone (DHT)- capable of promoting cellular growth and survival. DHT tethers AR with high affinity in the cytoplasm, replaces heat-shock proteins bound to the AR and promotes the interaction between the N and C termini stabilizing the AR dimer and slowing-down the rate of ligand dissociation [8]. The translocation into the nucleus takes place when the complex AR-DHT binds to importin-α [8]. Once inside the nucleus, the receptor dimerises and tethers to androgen response elements (AREs) within promoter regions of target genes, such as prostate-specific antigen (PSA) and transmembrane protease serine 2 (TMPRSS2). After recruiting other co-regulators in order to promote transcription, androgen receptor, dimers drive cell growth and survival [9–14].

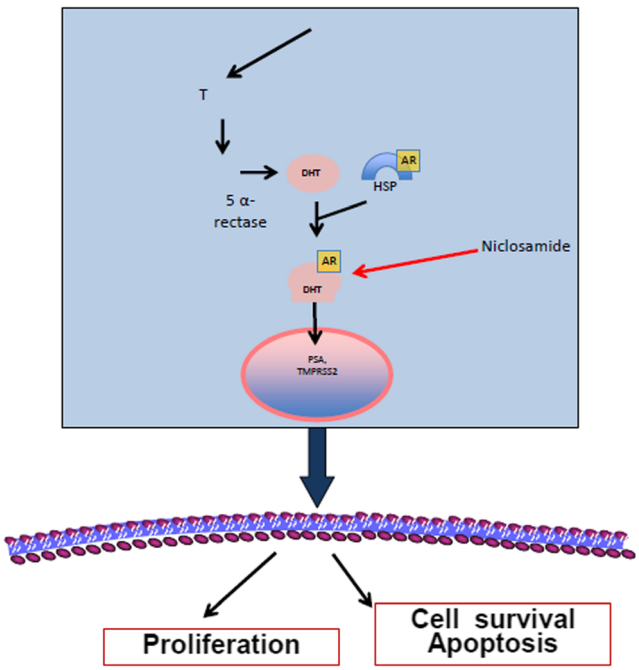

Fig. 1 The molecular pathway of niclosamide on full length AR and ARV7

At the structural level AR belongs to the steroid hormone group of nuclear receptors together with the mineralocorticoid receptor (MR), glucocorticoid receptor (PR), oestrogen receptor (ER) and progesterone receptor (PR). All these nuclear receptors are structurally similar and can have several interactions between themselves [12, 15–17]. AR gene is located at the chromosomal locus Xq11-Xq12 and the protein coding region has 2757 nucleotides [18–20]. The coding region is composed of eight exons and encodes a 110 kDa protein with 919 amino acids [21]. On a structural level, the AR is characterised by three domains, all of which are important for receptor function. Such domains are the N-terminal domain (NTD), followed by the DNA binding domain (DBD) and a flexible hinge region which connects the DBD region to the C-terminal ligand binding domain (LBD) [15]. Currently the main structural domains have been solved singularly. The NTD is entirely coded by exon 1 and represents most of the size of the AR (residues 1–555); it is highly variable in the human population and includes the activation function 1 (AF1, residues 142–485) [22–24]. This region is constitutively active and it is the primary effector region of the NTD [25]; AF1 contains two singular transcription units: Tau 1 (amino acids 23–27) and Tau 5 (amino acids 433–437) [26], both important in the interactions between the NTD and the LBD which, in turn, are crucial in regulating some androgen-dependent genes [27–29]. The DBD (residues 556–623) is the central domain of AR, is highly conserved among steroid hormone receptors and is encoded by exons 2 and 3. It is a cysteine-rich region and is followed by a hinge region (residues 624–665). Even though AR functions as a dimer, every DBD monomer has a core comprising two zinc fingers, each of which includes four cysteine residues that coordinate a zinc ion [30]. Through interaction with the major groove of the DNA helix, the zinc fingers are able to bind the androgen receptor to its target genes [14]. The nuclear localization signal (NLS) (residues 617–633) is situated at the junction between the DBD and the hinge region, which plays a pivotal role in the nuclear import of the receptor [31, 32]. The hinge region also plays a complex role in coactivator recruitment and DNA binding. Furthermore, such hinge is target for acetylation, methylation and ubiquitylation [33, 34]. The LBD (residues 666–919) has been first solved by crystallography in the year 2000 and it is structurally well-characterized. According to the crystal structure, the LBD structure is arranged in a three-layer fold and consists of eleven α-helices and four short β-strands creating two anti-parallel β-sheets. A ligand-binding pocket (LBP) is surrounded by N termini of H3, H5 and H11. H12 is a peripheral α-helix, whose folding on the upper part of LBP acts as a lid to close the agonist-binding, forms the core of the activation function 2 domain (AF-2) [35, 36]. Over the past decades, several groups have demonstrated that regulation of AR can occur at the protein level, involving post-translational modifications and

interactions with regulatory proteins, and at the genomic level, where some key mutations correlated with the PC have been identified [37–40]. Moreover, AR activity can be regulated by alternative splicing, but some ARVs that lack the LBD and enhance gene transcription without androgen hormones signalling [41]. Up-to-date, ARVs are considered responsible for AR activity, PC cell survival and tumour progression [37]. Within ARVs group, 7 splice version of the Androgen Receptor (AR-V7) is the only variant endogenously observed at the protein level and therefore well-characterized [5]. Many studies in literature have shown that AR-V7 is the predominant AR variant. Antonarakis ES et al. showed that ARV7 is highly expressed in circulating tumour cells (CTC), as quantified with RT-PCR [50], and it is associated with resistance to abiraterone and enzalutamide in metastatic CRPC patients [42–44]. AR-V7 is a truncated androgen-receptor protein lacking the C-terminal ligand-binding domain, but retaining the transactivating N-terminal domain. As a consequence it is unable to bind to ligands, but remains constitutively active as a transcription factor and it is therefore able to promote the activation of target genes promoting cancer [45, 46]. Figure 1 shows a model of action of androgens on the receptor in the context of new treatments aiming to inhibit both full length and AR-V7.

Niclosamide and its inhibitory role of AR-V7 in CRPC: Preclinical data

Niclosamide (5-Chloro-N-{2-chloro-4-nitrophenyl}-2-hydroxybenzamide) is an already FDA-approved anti-helminthic drug that has been identified as a potent AR-V7 inhibitor in prostate cancer cells. Niclosamide significantly downregulated AR-V7 protein level by protein degradation through a proteasome dependent pathway. Therefore its inhibition offers a valid approach for the treatment of those CRPC samples that are AR-V7-positive. In 2014, Liu et al. identified inhibitors of AR variants capable to overcome drug resistance conferred by AR-V7. Niclosamide inhibits androgen receptor variants expression and overcomes enzalutamide resistance in CRPC [55]. Liu et al. discovered that niclosamide is capable of decreasing protein levels of AR-V7 in CRPC cell lines, by means of ubiquitin proteasome system. They used the 26S proteasome inhibitor MG132 to inhibit proteosomal degradation. The authors showed that MG132 (5 μM) efficiently reduced the ability of niclosamide-mediated inhibition of AR-V7. The drug was able to reduce AR-V7 protein levels, while measurement with qPCR proved that the full length *AR-V7* mRNA remained unchanged, while Western Blotting showed lower levels of AR-V7 protein. The transcriptional activity of AR-V7 was noticeably down-modulated as proven by luciferase reporter gene assay, and recruitment of AR-V7 at the PSA promoter resulted in a diminution of PSA protein expression. Furthermore, by ELISA, the authors proved that no PSA levels were observed in cells treated with niclosamide. ChiP assays further confirmed that AR-V7 was recruited to the promoter. It was therefore the transcriptional activity of AR-V7 to be impaired in the presence of niclosamide. Moreover, as proven by cell-death ELISA, niclosamide fostered the AR-V7 positive cells to become apoptotic. The authors further proved in vitro and in vivo that niclosamide was capable of enhancing enzalutamide efficacy. Moreover, the authors proved that 25 mg/kg of niclosamide reduced tumour volume in mice xenografted with AR-V7 expressing CRPC cells. Additionally, the combination of niclosamide with enzalutamide was more efficient compared to niclosamide alone as it significantly reduced tumour volume compared to niclosamide on its own ($p < 0.05$). The authors concluded that niclosamide is a promising inhibitor of AR variants useful to treat patients with advanced PC, especially those resistant to enzalutamide. Interestingly it was shown that niclosamide restored sensitivity to enzalumatide by the inhibition of IL6-Stat3-AR axis, which is a crucial mechanisms of enzalutamide resistance [56]. In 2016, Liu C et al. demonstrated that niclosamide was a stronger agent when combined with abiraterone compared to single agents niclosamide or arbiraterone in AR-V7 positive CRPC cells. They showed how AR-V7 conferred resistance to their cell line. They knocked-down by siRNA the AR-V7 gene in AR-V7 positive cell lines CWR22RV1 and C4-2B MDVR and demonstrated that AR-V7 siRNA treated cell lines became sensitive to the abiraterone drug. On the same note, when the original abiraterone-resistant cell lines were treated with niclosamide they became sensitive to abiraterone treatment in both in vitro and in vivo models. This was first proven by showing that the cell numbers significantly decreased by niclosamide with or without abiraterone ($p < 0.05$). Clonogenic assays confirmed these results by showing how the niclosamide and/or abiraterone was/were able to significantly reduce the number of colonies in these cell lines ($p < 0.05$). The highest reduction in colony numbers, as for the in vivo tumour growth, was observed using the combination of niclosamide with abiraterone ($p < 0.05$). Also the colony size was reduced in the cell lines treated with these two compounds. As to the in vitro results the authors proved that when CWR22RV1 or C4-2B MDVR were treated with or without abiraterone the cell growth was inhibited and the combination had a synergic effect, with a significantly greater ability to inhibit cell-growth compared to single agents ($p < 0.05$) [57]. Finally, it has been recently demonstrated that targeting AR-V7 with niclosamide can resensitize bicalutamide-resistant cells to bicalutamide. Furthermore, combination of niclosamide with bicalutamide inhibits enzalutamide resistant tumor growth, suggesting that the combination of niclosamide and bicalutamide could be an interesting strategy to treat patients who fail to respond to enzalutamide therapy [58].

Table 1 Ongoing clinical trials with niclosamide in CRPC

Clinical trial identifier	Phase	Setting Administered	Primary endpoint	Status
NCT02409199 NCT03123978	II/III1	Second line Niclosamide + enzalutamide	PFS, DLT, RP2D	Recruiting
NCT02532114	1	Niclosamide + enzalutamide	Safety and Tolerability	Active, not recruiting
NCT02807805	2	Niclosamide+ Abiraterone acetate+ Prednisone	PSA response	Recruiting

Radiological Progression Free Survival; *PFS*, Dose Limiting Toxicity; *DLT*, phase II recommended dose (RP2D)

Niclosamide: On-going clinical data

There are three clinical trials testing niclosamide in CRPC patients: two of them use it in combination with enzalutamide (Phase I, NCT02532114; Phase I, NCT03123978); one of them uses it in combination abiraterone (Phase II, NCT02807805). NCT02532114 clinical trial's primary objective is testing the safety and tolerability of niclosamide in combination with enzalutamide. This is a phase I that uses the combination of niclosamide and enzalutamide as a second line of treatment after the CRPC patients had progressed on abiraterone acetate. NCT03123978 clinical trial's primary objective is to determine safety and the phase II recommended dose in the treatment of patients with CRPC. As secondary objectives PFS and response by PSA are measured. NCT02807805 is a phase II clinical trial is testing PSA-measured responsiveness as primary objective and as secondary objective the overall response rate, PFS and toxicity. Moreover, since niclosamide is specific to AR-V7, these trials may shed some light over the question of the effectiveness of targeting only the AR-V7 in CRPC. To obtain always more statistically robust data, successively the first obtained results should be further tested in larger and randomized clinical trials after passing all the other initial phases of the clinical trials. The clinical trials testing niclosamide are summarized in Table 1.

Future directions

Despite the progress in this field, CRPC still remains one of the most lethal diseases. The AR-V7 isoform, which is often found in CRPC, it is very difficult to treat by the standard second-generation anti-androgens because this variant has the peculiarity of being constitutively active and therefore independent of the binding of androgens for its activation and downstream signalling. Towards the adoption of always more efficient and target-specific treatments, the scientific community has closely been looking at the efficacy in of CRPC therapies directed against the AR-V7-positive CRPC for its degradation. There has been an interest for the use of niclosamide for the treatment of patients with this type of receptor variant after the positive results of the first pre- and clinical data. The results of first ongoing clinical trials could prove crucial for the further development of niclosamide in later stages clinical trials.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent For this type of study, formal consent is not required.

References

1. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68:7–30
2. Stein MN, Jang TL (2016) Striving toward a cure for prostate Cancer. J Clin Oncol 34:2075–2078
3. Schweizer MT, Yu EY (2015) Persistent androgen receptor addiction in castration-resistant prostate cancer. J Hematol Oncol 8:128
4. Kantoff PW et al (2010) Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer. Engl J Med 3635
5. Wadosky KM, Koochekpour S (2017) Androgen receptor splice variants and prostate cancer: from bench to bedside. Oncotarget 8: 18550–18576
6. Rosner W, Hryb DJ, Khan MS, Nakhla AM, Romas NA (1991) Sex hormone-binding globulin: anatomy and physiology of a new regulatory system. J Steroid Biochem Mol Biol 40:813–820
7. Baker ME (2002) Albumin, steroid hormones and the origin of vertebrates. J Endocrinol 175:121–127
8. Srinivas-Shankar U, Wu FC (2006) Drug insight: testosterone preparations. Nat Clin Pract Urol 3:653–665
9. Shang Y, Myers M, Brown M (2002) Formation of the androgen receptor transcription complex. Mol Cell 9:601–610
10. Dehm SM, Tindall DJ (2006) Molecular regulation of androgen action in prostate cancer. J Cell Biochem 99:333–344
11. Wang Q, Carroll JS, Brown M (2005) Spatial and temporal recruitment of androgen receptor and its Coactivators involves chromosomal looping and polymerase tracking. Mol Cell 19:631–642

12. van Royen ME, van Cappellen WA, de Vos C, Houtsmuller AB, Trapman J (2012) Stepwise androgen receptor dimerization. J Cell Sci 125:1970–1979
13. Heinlein CA, Chang C (2004) Androgen receptor in prostate Cancer. Endocr Rev 25:276–308
14. Beato M, Herrlich P, Schütz G (1995) Steroid hormone receptors: many actors in search of a plot. Cell 83:851–857
15. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM (1995) The nuclear receptor superfamily: the second decade. Cell 83:835–839
16. Tsai M, O'Malley BW (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451–486
17. Nuclear Receptors Nomenclature Committee (1999) A unified nomenclature system for the nuclear receptor superfamily. Cell 97: 161–163
18. Lubahn DB et al (1988) Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science 240:327–330
19. Brown CJ, Goss SJ, Lubahn DB, Joseph DR, Wilson EM, French FS, Willard HF (1989) Androgen receptor locus on the human X chromosome: regional localization to Xq11-12 and description of a DNA polymorphism. Am J Hum Genet 44:264–269
20. Migeon BR, Brown TR, Axelman J, Migeon CJ (1981) Studies of the locus for androgen receptor: localization on the human X chromosome and evidence for homology with the Tfm locus in the mouse. Proc Natl Acad Sci U S A 78:6339–6343
21. Gelmann E, Molecular P (2002) Biology of the androgen receptor. J Clin Oncol 20:3001–3015
22. Sasaki M, Kaneuchi M, Sakuragi N, Fujimoto S, Carroll PR, Dahiya R (2003) The polyglycine and polyglutamine repeats in the androgen receptor gene in Japanese and Caucasian populations. Biochem Biophys Res Commun 312:1244–1247
23. Hsing AW, Gao YT, Wu G, Wang X, Deng J, Chen YL, Sesterhenn IA, Mostofi FK, Benichou J, Chang C (2000) Polymorphic CAG and GGN repeat lengths in the androgen receptor gene and prostate cancer risk: a population-based case-control study in China. Cancer Res 60:5111–5116
24. Chang CS, Kokontis J, Liao ST (1988) Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. Proc Natl Acad Sci U S A 85:7211–7215
25. McEwan IJ (2004) Molecular mechanisms of androgen receptor-mediated gene regulation: structure-function analysis of the AF-1 domain. Endocr Relat Cancer 11:281–293
26. Callewaert L, Van Tilborgh N, Claessens F (2006) Interplay between two hormone-independent activation domains in the androgen receptor. Cancer Res 66:543–553
27. Doesburg P, Kuil CW, Berrevoets CA, Steketee K, Faber PW, Mulder E, Brinkmann AO, Trapman J (1997) Functional *in Vivo* interaction between the amino-terminal, transactivation domain and the ligand binding domain of the androgen receptor †. Biochemistry 36:1052–1064
28. He B, Kemppainen JA, Wilson EM (2000) F *XX* LF and W *XX* LF sequences mediate the NH_2 -terminal interaction with the ligand binding domain of the androgen receptor. J Biol Chem 275:22986–22994
29. Wilson EM (2011) Analysis of Interdomain Interactions of the Androgen Receptor. Methods in molecular biology (Clifton, N.J.) 776:113–129
30. Shaffer PL, Jivan A, Dollins DE, Claessens F, Gewirth DT (2004) Structural basis of androgen receptor binding to selective androgen response elements. Proc Natl Acad Sci 101:4758–4763
31. Jenster G, Trapman J, Brinkmann AO (1993) Nuclear import of the human androgen receptor. Biochem. J 293(Pt 3):761–768
32. Zhou ZX, Sar M, Simental JA, Lane MV, Wilson EM (1994) A ligand-dependent bipartite nuclear targeting signal in the human androgen receptor. Requirement for the DNA-binding domain and modulation by NH2-terminal and carboxyl-terminal sequences. J. Biol. Chem 269:13115–13123
33. Haelens A, Tanner T, Denayer S, Callewaert L, Claessens F (2007) The hinge region regulates DNA binding, nuclear translocation, and transactivation of the androgen receptor. Cancer Res 67:4514–4523
34. Clinckemalie L, Vanderschueren D, Boonen S, Claessens F (2012) The hinge region in androgen receptor control. Mol Cell Endocrinol 358:1–8
35. Matias PM, Donner P, Coelho R, Thomaz M, Peixoto C, Macedo S, Otto N, Joschko S, Scholz P, Wegg A, Bäsler S, Schäfer M, Egner U, Carrondo MA (2000) Structural evidence for ligand specificity in the binding domain of the human androgen receptor. J Biol Chem 275:26164–26171
36. Tan MHE, Li J, Xu HE, Melcher K, Yong E (2015) Androgen receptor: structure, role in prostate cancer and drug discovery. Acta Pharmacol Sin 36:3–23
37. Wadosky KM, Koochekpour S (2016) Molecular mechanisms underlying resistance to androgen deprivation therapy in prostate cancer. Oncotarget 7:64447–64470
38. Koochekpour S (2010) Androgen receptor signaling and mutations in prostate cancer. Asian J Androl 12:639–657
39. Heemers HV, Tindall DJ (2007) Androgen receptor (AR) Coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. Endocr Rev 28:778–808
40. Van der Steen T, Tindall D, Huang H (2013) Posttranslational modification of the androgen receptor in prostate Cancer. Int J Mol Sci 14:14833–14859
41. Dehm SM, Tindall DJ (2011) Alternatively spliced androgen receptor variants. Endocr Relat Cancer 18:R183–R196
42. Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, Chen Y, Mohammad TA, Chen Y, Fedor HL, Lotan TL, Zheng Q, de Marzo AM, Isaacs JT, Isaacs WB, Nadal R, Paller CJ, Denmeade SR, Carducci MA, Eisenberger MA, Luo J (2014) AR-V7 and resistance to Enzalutamide and Abiraterone in prostate Cancer. N Engl J Med 371:1028–1038
43. Steinestel JAM, Luedeke M, Arndt A (2014) Detecting predictive androgen receptor modifications in circulating prostate cancer cells. Oncotarget. https://doi.org/10.18632/oncotarget.3925
44. Scher HI, Graf RP, Schreiber NA, McLaughlin B, Lu D, Louw J, Danila DC, Dugan L, Johnson A, Heller G, Fleisher M, Dittamore R (2017) Nuclear-specific AR-V7 protein localization is necessary to guide treatment selection in metastatic castration-resistant prostate Cancer. Eur Urol 71:874–882
45. Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ (2008) Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate Cancer therapy resistance. Cancer Res 68:5469–5477
46. Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, Han M, Partin AW, Vessella RL, Isaacs WB, Bova GS, Luo J (2009) Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate Cancer. Cancer Res 69:16–22